THE REDUCTION DIVISION IN THE ANTHERS OF HYACINTHUS ORIENTALIS.*

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There are many problems in connection with the reduction division, which have not been satisfactorily solved. It was, therefore, determined to take up the problem on some plant which would show the nuclear structures as distinctly as possible. The plant finally selected for the investigation was the common hyacinth, Hyacinthus orientalis L.

The dry bulbs of the common hyacinth were planted in the greenhouse at various intervals during the Fall of 1907. A bulb was opened and examined almost every day, but it was not until the first week in November that the desired stages were secured, since it was some time before it was ascertained how long the dry bulbs must remain in the ground before reduction begins. This depends entirely on the time of planting. If the bulbs are put in the ground early in the fall it will be some time before reduction takes place. The desired stages were finally secured in bulbs which were planted in the last week of October, and which remained in the ground less than a week, showing that slow development takes place in the bulb while in the dry state. All of the material killed on the days from the 1st to the 4th of November showed the various reduction stages, and even in the individual bulb nuclei ranging from early microsporocytes to tetrads were found. The usual methods of killing and imbedding were used and the sections were cut from 10–12 mic. thick. The slides were stained most satisfactorily with Delafield's Haemotoxylin. This study was begun under the direction of Prof. R. F. Griggs and was completed under Prof. J. H. Schaffner, to both of whom the writer is greatly indebted for kindly assistance and suggestions.

In the younger anthers many nuclei were found showing the last division previous to the formation of the microsporocytes (figs. 1, 2). In these nuclei the spirems showed a linin thread with definite granules. There is evidently no long resting stage in the sporocyte previous to reduction and this makes it difficult to recognize the young sporocytes from their mother cells, the two being present in the sporangium at the same time. A tendency toward indefinite massing of the chromatin (figs. 3, 4) in the early sporocytes, gradually disappears as the threads of the network become more prominent (fig 5). There does not, however, seem to be any definite massing of the chromatin into

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recognizable protochromosomes as in Thalictrum, Agave, and various other plants.

Following this stage the complicated spirem is formed, which gradually thickens until its continuity can be traced with comparative ease, although it is very irregularly looped and twisted (fig. 9). At this stage the so-called synizesis probably occurs. This varies from slight contraction to a dense massing in the center or side of the nuclear cavity. The nucleolus may or may not appear free from the mass (fig. 8). Sometimes series of sections, apparently of the proper stage, do not show synizesis, while in others contractions are present even in well developed spirems.

The spirem continues to thicken and the irregular twisting and winding becomes less complicated, with a strong tendency to form loops, the crossing threads producing a dense mass in the center (figs. 10, 11). This arrangement of the loops with crossing in the center has been noted in other members of the lily family.

No trace could be found of double granules or a split in the spirem. This, however, may have been due to the staining. The linin and chromatin granules appeared quite distinct in the earlier stages (figs. 5a, 9a) but with the twisting and winding prior to the formation of loops, they stain much darker and more uniformly until differentiation finally disappears. The twisting of the spirem now becomes gradually simplified until there are approximately eight loosely rounded loops, the eight incipient chromosomes, corresponding to the reduction number of chromosomes (figs. 12,13). These loops thicken and condense until there are clearly eight loops radiating from the center, with the looped ends pointing outward toward the nuclear wall (figs. 14, 15). The eight chromatin loops were distinctly seen and counted a large number of times.

The arrangement of the spirem into loops, corresponding in number to the reduced number of chromosomes and their subsequent separation and massing to form the chromosomes was mentioned by Schaffner in his paper on Lilium philadelphicum. He found that the "twisted chromatin band arranges itself so as to form twelve loops, the heads of the loops being close to the nuclear membrane." This arrangement was also found by Brown in his study of the embryo-sac of Peperomia. In this case he finds that after the looping becomes more pronounced, the spirem segments, each loop giving rise to a chromosome. He draws the conclusion that chromosomes are, therefore, formed by the coming together, side by side, of parts of the spirem that before were arranged end to end. Gates notes a somewhat similar condition in Oenothera rubrinervis, in which

he finds the spirem varying in thickness in different parts, exhibiting constrictions and dilations indicating more or less clearly where segmentation into chromosomes will take place. He says there is nothing to indicate that the successive chromosomes are members of a pair, but one chromosome frequently swings around and pairs with its neighbor on the skein. He concludes: "We do not really have, then, a transverse division of chromosome bivalents, but a separation of whole (somatic) chromosomes."

One of the most interesting and prominent features in the reduction karyokinesis of the hyacinth is the marked individuality of the bivalent chromosomes. After the formation of the loops, a transverse breaking of the continuous spirem takes place by which they are separated (fig. 16.) The contracting loops show a tendency toward definite size and shape and when the chromosomes have their final form they show a striking individuality. There are two comparatively small chromosomes, a third of medium size and another only a little larger, while the remaining four are of giant proportions when compared with the two smallest. Of the two medium sized chromosomes, one is generally somewhat heart- or v- shaped, while the other is a more or less irregular mass usually without two projecting limbs. Of the four large ones, two in favorable sections, always show a prominent twist while the other two show a more compact and regular form. These shapes and sizes were noted in many different nuclei (figs. 18–22).

Fine threads were often present, extending from the loops to the nuclear wall or connecting the loops themselves. These threads were also present after the separation into chromosomes (fig. 17).

The formation of the spindle and the subsequent stages were not included in the study.

It will be evident from an examination of the figures, that the pairing which takes places in the formation of the bivalent or reduction chromosomes, is between univalents of essentially the same shape, size and activity. There can be little doubt but that these similar conjugating chromosomes represent a pair, the one maternal and the other paternal. The equivalent maternal and paternal chromosomes are, therefore, of essentially corresponding shapes and sizes. To determine definitely by observation whether all the maternal or paternal chromosomes go to a single pole, will require cases in which a difference in character between the two can be determined.

SUMMARY.

1. The chromatin network of the resting nucleus is transformed into a continuous spirem with no definite evidence of the presence of protochromosomes.

2. No spliting or doubling of the linin thread or of the chromatin granules was observed; the chromosomes are apparently arranged end to end in the spirem.

3. Synizesis appeared at various stages but was apparently not constant for any particular stage.

4. The continuous spirem shortens and twists into 8 loops, radiating outward toward the nuclear wall, the crossing threads forming a central knot.

5. The 8 loops break apart at the center to form the 8 bivalent chromosomes.

6. Fine connecting strands often appear between the chromosomes and the nuclear wall or between the chromosomes themselves.

7. The chromosomes show a striking difference as to shape and size.

8. The pair of univalent chromosomes, which must have united to form a bivalent chromosome are alike in shape and size and apparently represent maternal and paternal bodies.

LITERATURE.

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EXPLANATION OF PLATE XXXII.

Fig. 1. Mother cell of microsporocytes.
Fig. 2. The same.
Fig. 3. Microsporocyte with chromatin network and showing irregular masses.
Fig. 4. Microsporocyte with delicate linin threads and prominent chromatin granules.
Fig. 5. Microsporocyte with expanding nucleus and more prominent threads.
Fig. 5a. Single threads showing rows of chromatin granules.
Fig. 6. Threads of network adjusting themselves into a definite spirem.
Fig. 7. Spirem becoming thicker.
Fig. 8. Synizesis stage; massing of chromatin.
Fig. 9. Spirem showing irregular twisting.
Fig. 9a. Single threads of same stage.
Fig. 10. Spirem beginning to be thrown into definite loops, the threads crossing and massing in the center.

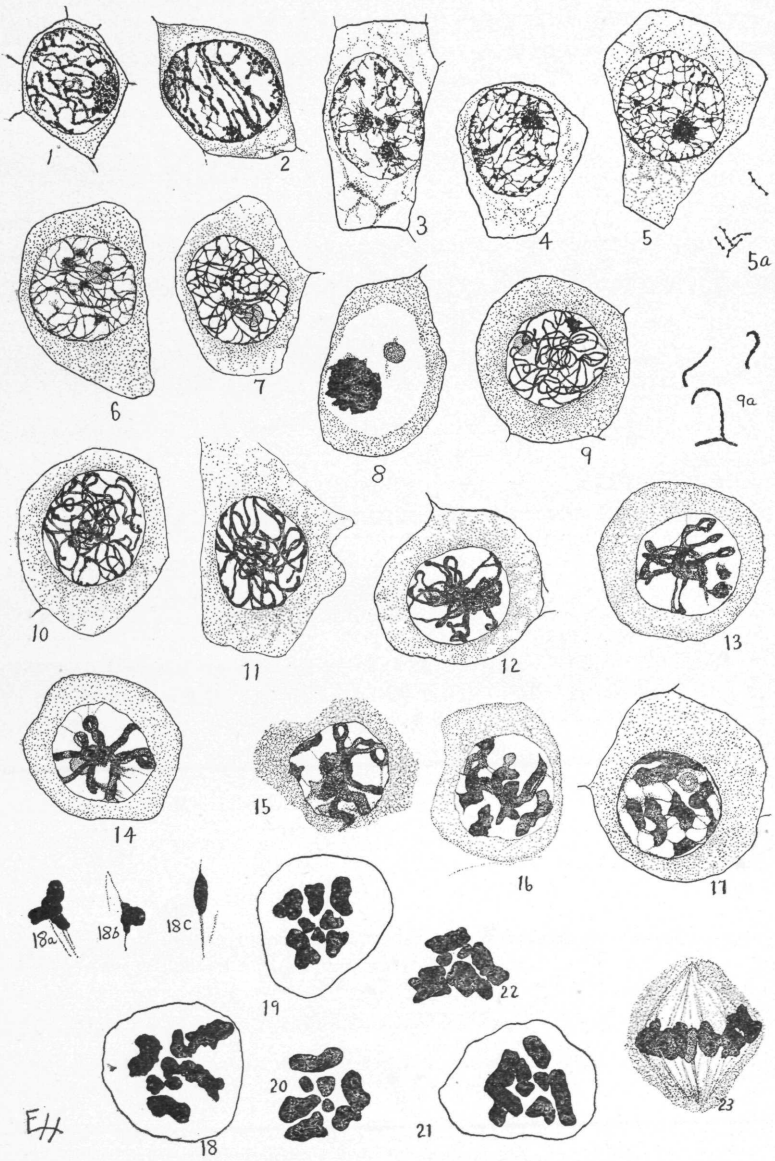

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Fig. 11. Thicker spirem with loops becoming more definite.
Fig. 12. Spirem adjusting itself into approximately 8 loops.
Fig. 13. Loops more condensed than in Fig. 12.
Fig. 14. The 8 loops distinctly formed.
Fig. 15. Nucleus showing details of two loops.
Fig. 16. Microsporocyte after separation of the loops, before much contraction has taken place.
Fig. 17. Further contraction of chromosomes; connecting threads present.
Fig. 18. Chromosomes beginning to show individuality before complete condensation has taken place.
Figs. 18a, b, c. Types of chromosomes as arranged on spindle.
Figs. 19–22. Chromosome groups showing individual shapes; two large twisted ones, two small ones, and two of medium size, one of which is heart-shaped, also two large ones with no definite shape.
Fig. 23. The chromosomes drawn into the equatorial plane.